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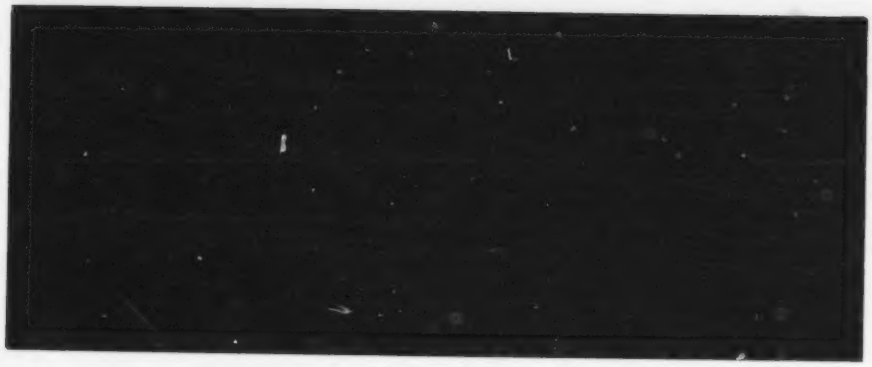

Final Report

2.4-32

Embryo Transfer Technology in Game Farm Animals

Conducted by

Marimba Farm Inc.

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EMBRYO TRANSFER TECHNOLOGY IN GAME FARM ANIMALS

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Abstract

During the last six months we have attempted to train a small group of bison cows to regular handling, and to collect embryos from the females and eventually transfer them to domestic cattle. This required us to synchronize & superovulate the cows, and collect, and transfer embryos. We have been able to synchronize the animals, and also sometimes superovulate them. We still face the problem of collecting viable embryos, although we have been able to collect unfertilized eggs. Much work remains to be done before this becomes a viable economic option for the bison rancher.

Technical Report

A superovulation program has been designed for the bison based upon abundant available information for the domestic cow and limited information and experience on previous bison programs at the Western College of Veterinary Medicine and Marimba Farm. This program has included study of the estrous cycle, endocrinology, estrus synchronization, superovulation and embryo collection methodology. I also consulted with the only other group studying bison embryo transfer when I traveled to College Station, Texas, during a trip in late October 1989 which was sponsored by a federal TIP grant.

The information is obtained from numerous reports of the use of gonadotrophin preparations in cattle. These include porcine FSH (FSH-P, Schering Corporation), which has both FSH and LH activity; FSH rich preparation with low LH activity (Folltropin, Vetrapharm); Equine chorionic gonadotrophin (ECG) or pregnant mare serum gonadotrophin (PMSG); and menopausal gonadotrophin (Pergonal - HMG, Serono Labs). The dose schedules used with FSH-P, Folltropin or HMG in cattle superovulation programs require 4 or 5 day regimes of two treatments daily in order to produce adequate response. PMSG on the other hand has been used as a single injection but has been shown to persist in the animal system for prolonged periods, and is also subject to some degree of biological variation, and therefore can create variable response in subject animals.

It has been shown in cattle that stress induced situations can decrease follicular growth significantly, which results in poor superovulatory response. Bison are less amenable to handling in a chute system than cattle. We were able to train the bison into the handling yards by regular feeding and handling through the system which occurred throughout the trials, and was initiated as soon as the corrals had been constructed. Even after this training it was evident that the bison be come excitable in the chutes and would develop a reluctance to enter the corrals voluntarily, even for food. Therefore we decided that a superovulation and synchronization system that involved the minimum amount of handling was essential. This basically ruled out a twice daily injection regime.

Our first trials involved the use of PMSG together with a monoclonal antibody (MoAb) which was designed to overcome the undesirable prolonged effects of this preparation. The protocol was as follows:-

Day 0	Estrus
Day 9 am	2500 i.u. PMSG
Day 11 am	750 µg Estrumate
Day 13	Estrus, natural mating + 3000 units PMSG MoAb
Day 19	Embryo collection

We carried out this regime in September and October 1989. Our results did not yield any viable embryos, but we did gather a variety of information that had not previously been recorded in the bison. This included palpation findings, hormonal changes, estrus behaviour both in the natural cycle and the superovulated cycle, synchronization techniques and embryo collection techniques. These are discussed in greater detail below.

We had also initiated a further synchronization regime in November which we had to abandon because of the unfortunate death of Dr. Pawlyshyn's father.

As yet unpublished information that has been gleaned by Dr. Mapeltoft and his graduate students at the WCVM was applied in December. In a preliminary study of 31 cows they found that a single injection of a high dose of Folltropin together with a low dose of PMSG has produced acceptable superovulatory response with no significant differences in the number of transferable embryos when compared to a 4 day regime of FSH injections. This obviously has potential application in the bison project as it involves a minimum number of handling events. The protocol is as follows:-

Day 0	Estrus
Day 9 am	18 mg Folltropin + 750 iu PMSG
Day 11 am	750 µg Estrumate
Day 12	Heat observation initiated
Day 13	Estrus
Day 19	Embryo collection

In December The use of this regime resulted in a very poor superovulatory response. The factors involved are not totally clear. One fact that did emerge was that palpation findings did not match the endocrinology profile. Low progesterone concentrations were found at the time that we started the the superovulation program, despite the fact that a corpus luteum was palpated. It is possible that this was related to premature luteal regression, which has been seen in the cow. Hormonal information, other than our own, regarding any aspect of the estrus cycle of the bison is non-existent, so that it is difficult to be certain of the factors involved.

Another confounding factor in December was the bitterly cold weather which frustrated our attempts as solutions could not be prevented from freezing and working conditions became unreasonable. We decided to leave the animals alone during January and early February in the hope that the weather would have improved sufficiently to allow us to get going again.

We have carried out several activities in March. We attempted to superovulate and collect the bison cows on two occasions . I attended a game farming conference in Loveland, Colorado, at the invitation of the Colorado Elk & Game Breeders Association, and had a lengthy discussion with a bison breeder who has been cooperating with the Texas A & M team on their bison embryo transfer project. We had set up the bison for an embryo collection attempt after a morning spent examining them. Despite almost continuous observation they did not come into heat as expected, and we rescheduled the attempts at a later date. We were again able to manage the animals, but not successful in the collection of any embryos. Both the delay in heats, and the failure to collect embryos can probably be explained in terms of things learned at the end of the month in Colorado.

The delayed heats may be a feature of stresses related to handling, which is a problem that we had already identified, and that has received some attention in terms of modification of the handling systems and the building of a small darkened shed over the chute system. This will hopefully accomplish two things. It will help the animals to accept the handling with greater equanimity, and also overcome the drug freezing problems described in the interim report.

The second failure, in terms of the collection of unfertilized embryos, may be a function of emerging information from the Colorado group. It would seem that some bison bulls are infertile in February and March. The data are a long way from being conclusive, and we do have evidence from a local zoo that this may not be an entirely simple answer. One bison cow at the Forestry Farm Park in Saskatoon gave birth to a calf in December, which would indicate conception either in late March or early April. There are no hard data of either semen collection or post-mortem studies to confirm or deny this matter, and it remains an important area for research. Our current plan is to delay the collection for about 6 weeks and start a regular training program for the animals as soon as the summer student for the project starts work at the beginning of May. This part of the continuing research will be funded from sources other than an ERDA grant, particularly from Marimba Farm itself.

Some specific detail of findings is outlined below:-

Palpation findings.

The major problem with palpation was the behaviour of the bison in chutes. Uterine anatomy is essentially the same as that of domestic cattle. The non-cyclic ovary is somewhat smaller, but follicles and corpora lutea could readily be identified.

Hormonal changes.

There has been no published information on seasonal patterns of reproduction in bison. The majority of bison calves are born in the spring and early summer, following a gestation of eight and a half months and a peak breeding period in June and early July. Nothing is known of the hormonal changes throughout the year, and we have been able to show that some degree of activity was present throughout our study period. Hormonal studies will continue and further elucidate this fascinating aspect of reproductive biology. Our data are as yet inconclusive, largely due to the small number of animals involved, but another year of work will hopefully help to clear up some of the questions and provide at least preliminary information.

Estrus behaviour.

Heat detection in bison involved periodic observation prior to and throughout the expected day of estrus. In non-superovulated animals the behaviour was much as has been described in this species. Bulls spend most of the time within 5 metres of the cow in heat, creating a more or less temporary monogamy. Grunting communication takes place periodically throughout the estrus period.

The female activity in superovulated animals is intensified. She shows tail elevation, swollen vulva, and mucus discharge. The male repeatedly shows Flehmen and associates more closely with the female, emitting numerous grunts.

During our observation periods no mounting was seen. We carried out observations of 2, 4 and 6 hour duration. The bull tried to mount on numerous occasions, but the cows would not stand. We may need to carry out 24 hour observations if natural breeding remains the most viable option. We are considering artificial insemination for future use.

Synchronization techniques

Previous, unpublished work indicated that we could synchronize bison using a "Synchromate B" regime, but this involved more handling than the one tried in the trials described above. We have been consistently able to synchronize bison, and induce estrus, using prostaglandin PGF2a. Until other information becomes available this will probably remain our method of choice. It lends itself to the potential for injection of animals by blow-gun or other remote method, further reducing the handling stress.

Embryo collection techniques

These appear to be very similar to those for cattle.

Problems

The most disappointing result was that we failed to collect any viable embryos. In the superovulated cow one can expect a fertilization rate of about 70%. The failure was most probably due to non-breeding in the animals. Consultation with the group at Texas A&M revealed that they have had exactly similar problems, but there is no other published information. The non-breeding may possibly be related to improper doses of drugs, which although well established for cattle, may require subtle changes when applied to bison. We have been provide, by the Texas group, with some preliminary data on their own trials, and these will be tested in our further work.

Semen Collection & AI.

One possible solution to the non-fertilization may be the use of artificial insemination. Our own results, and those of the group from Texas A&M indicate that bison semen is relatively easy to collect. On the other hand we have been unable to successfully freeze it, and the Texas group have had similar results. Given this information there exists the possibility for use of fresh bison semen in the current trials, as well as a new field in the investigation of freezing regimes for bison semen.

Summary & Conclusions

To date valuable information has been gained in estrous behaviour, synchronization, embryo collection, and endocrinology. We realize that the data are presently limited, but each succeeding program has the potential to add useful information. One of the limitations of the study is the rather small number of animals available for investigation. This is something about which can do very little, and we hope to be able to continue the studies with the present group. We have essentially only been working with the program for 6 months, and during that time we have been able to make several useful findings.

We plan to continue to compare the two superovulation regimes. One of the limitations here is that the monoclonal antibody for the PMSG is in rather short supply, but we hope to have enough to continue to work with this regime. We also plan to study the effects of superovulation on different days of the cycle, as the information in the domestic cow indicate that days 8,9, and 10 are the best days.

c) **Non-technical report**

Based upon our own limited results,and the data that has emerged from the study being conducted by Texas A & M, it would appear that bison are not simply a type of cow to which the embryo transfer technology learned in cattle can be applied. That some of the techniques apply is not surprising, but there are some that will need considerable refining before embryo transfer in this species becomes a useful economic option. The results in this brief trial, and from previous work indicate that viable embryo can indeed be collected. Successes to date have taken place when bottle raised bison females were used, and it may be that stress of handling plays an important role in this story. The study has raised more questions than it has answered and we need more information than is presently available in order to unravel the puzzle. I would not recommend this technique to bison farmers until more of the problems have been ironed out.

d) No publications have been forthcoming

e) Expenditures

Most of the salary component was found from another grant. Expenditures are shown in Appendix 1

f) Personnel & time commitment as a percentage of their full time.

Individual	*Role*	*Percentage of time (est)*
J.C.Haigh	Maintenance / Technical	0.5
J.M.Haigh	Maintenance	0.025
V. Pawlyshyn	Consultant	0.15
M. Braithwaite	Technical	0.01
G. Krebs	Technical	0.001
G. Steuwe	Technical	0.02
D. Hunter	Construction	0.01

g) Equipment purchases

Bison chute, as above
Consumables eg syringes, lumber

g) Other

We intend to pursue the research for a further year using some of our own R & D funding, as well as funds from a federal grant. Meanwhile, a copy of a magazine article published in the USA is enclosed. The article is about some slight successes in the bison AI field, and the farmer is one of the men thast I met in Loveland, Colorado.